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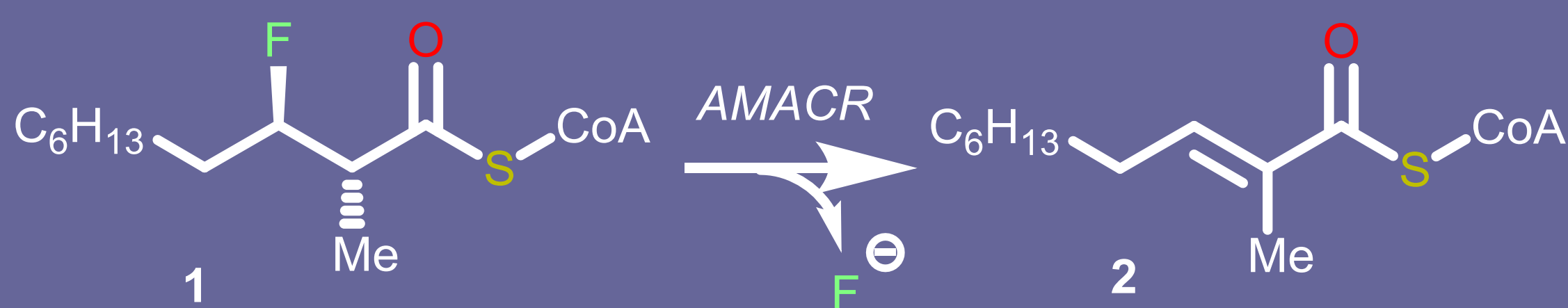
Maksims Yevglevskis,^a Guat Lee,^a Jenny Sun,^{a,b} Shiyi Zhou,^{a,b} Xiaolong Sun,^c Gabriele Kociok-Köhn,^c Tony D. James,^c Timothy J. Woodman^a and Matthew D. Lloyd^{a,‡}

Medicinal Chemistry, Department of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, U.K. [‡]email: M.D.Lloyd@bath.ac.uk
Department of Pharmacy, Shandong University, Peoples Republic of China
Department of Chemistry, University of Bath, Claverton Down, Bath BA2 7AY, U.K.

Introduction

Branched-chain fatty acids are common in the diet and are found in medicines such as Ibuprofen and related drugs. Metabolism of branched-chain fatty acids requires that the centres bearing the methyl groups possess *S*-stereochemical configuration, but centres with *R*-configuration are produced in the body and are found in the diet. Similarly, Ibuprofen and related drugs require *S*-configuration for anti-inflammatory activity, but these drugs are often given as a mixture of isomers. The enzyme α -methylacyl-CoA racemase (AMACR) catalyses *R*- to *S*- conversion of 2-methylacyl-CoA derivatives of fatty acids, enabling β -oxidation. Similarly, acyl-CoA derivatives of Ibuprofen and similar drugs undergo *R*- to *S*- conversion, resulting in pharmacological activation.^{1,2}

AMACR levels are increased in all prostate cancers, some colon cancers and other cancers.¹⁻³ In prostate cancer, higher AMACR levels results in higher rates of proliferation⁴ and androgen-independent growth⁵ and the enzyme is recognised as a novel drug target. However, few inhibitors have been identified, largely due to the difficulties in measuring enzyme activity which makes it difficult to quantify drug potency.¹ Recently, we showed that AMACR is able to catalyse the irreversible elimination of hydrogen fluoride from 3-fluoro-2-methyldecanoyl-CoA⁶ (Scheme 1).



Scheme 1: The AMACR catalysed elimination reaction.

This new reaction allows easier measurement of AMACR activity by ¹H NMR since the signals for the substrate and product 2-methyl groups appear in different places in the spectrum.⁶ The reaction can potentially be adapted for colorimetric or fluorescent measurement of AMACR activity by the use of chromogenic substrates or fluoride sensors. This study³ focuses on using the elimination reaction to measure drug potency and investigates development of a colorimetric or fluorometric assay.

Results and Discussion

Recombinant human AMACR 1A was pre-incubated with a number of known AMACR substrates or inhibitors before addition of 3-fluoro-2-methyldecanoyl-CoA substrate **1** at 100 μ M final concentration. After 1 hour incubation at 30° C the reaction was terminated by heating and conversion of **1** determined by ¹H NMR. All known substrates and inhibitors reduced conversion of **1** compared to positive controls (Table 1).³

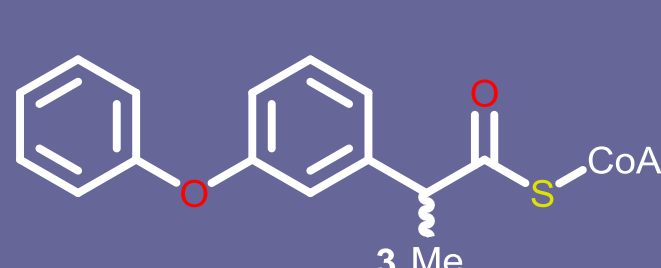
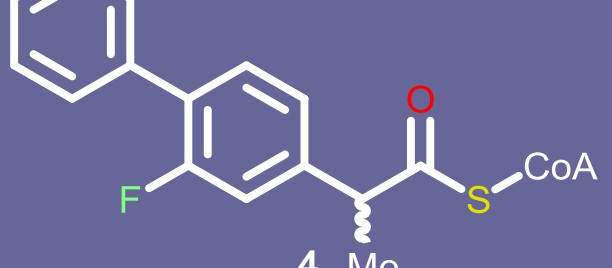
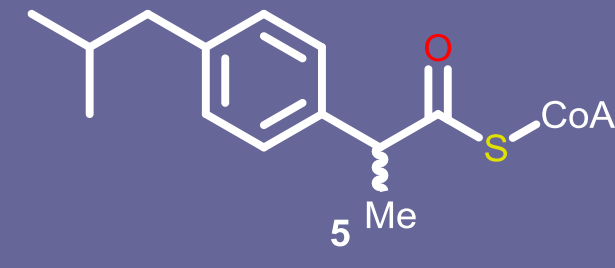
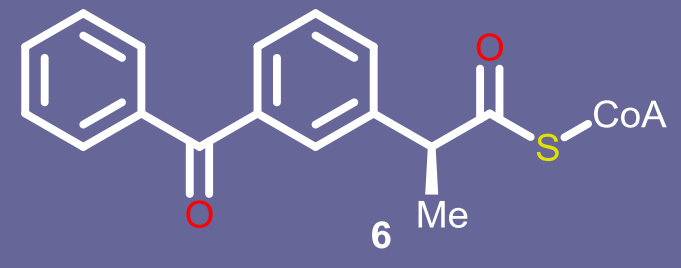
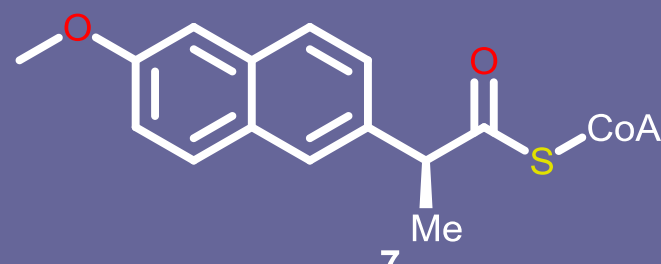
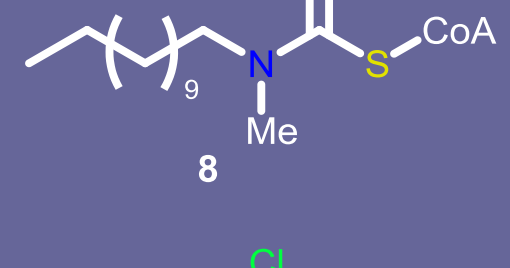
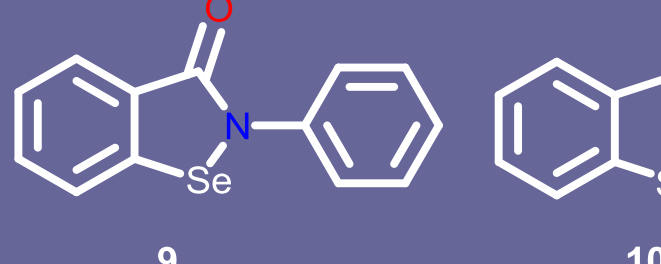
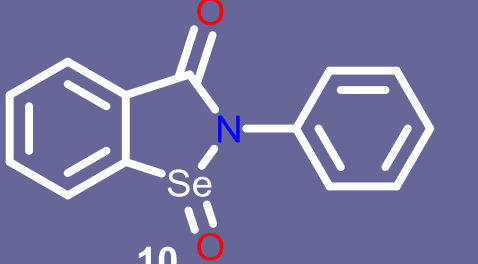
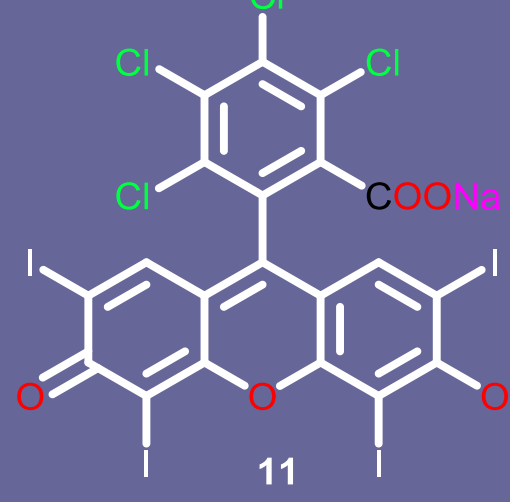
Inhibitor	Relative conversion in presence of inhibitor	Relative reduction compared to no inhibitor
	None	100%
	3	74.9 \pm 0.4%
	4	91.6 \pm 2.0%
	5	74.1 \pm 7.2%
	6	92.0 \pm 8.6%
	7	88.8 \pm 4.0 %
	8	16.8 \pm 1.8%
	9	<5%
	10	88.2 \pm 5.4%
	11	<5%

Table 1: Inhibition of AMACR activity by known substrates and inhibitors. Results are normalised to conversion levels in positive controls in order to enable ranking of inhibitors. Values are means (n = 2) \pm SD.

The presence of known substrates **3-7** in the assay all resulted in modest reductions in conversion of **1**, due to competitive inhibition. In contrast, inhibitor **8** almost completely abolished conversion of **1**, consistent with it been a known potent inhibitor (Figure 1). Protein modifying reagents **9-11** also reduced activity, in some cases abolishing enzyme activity.³

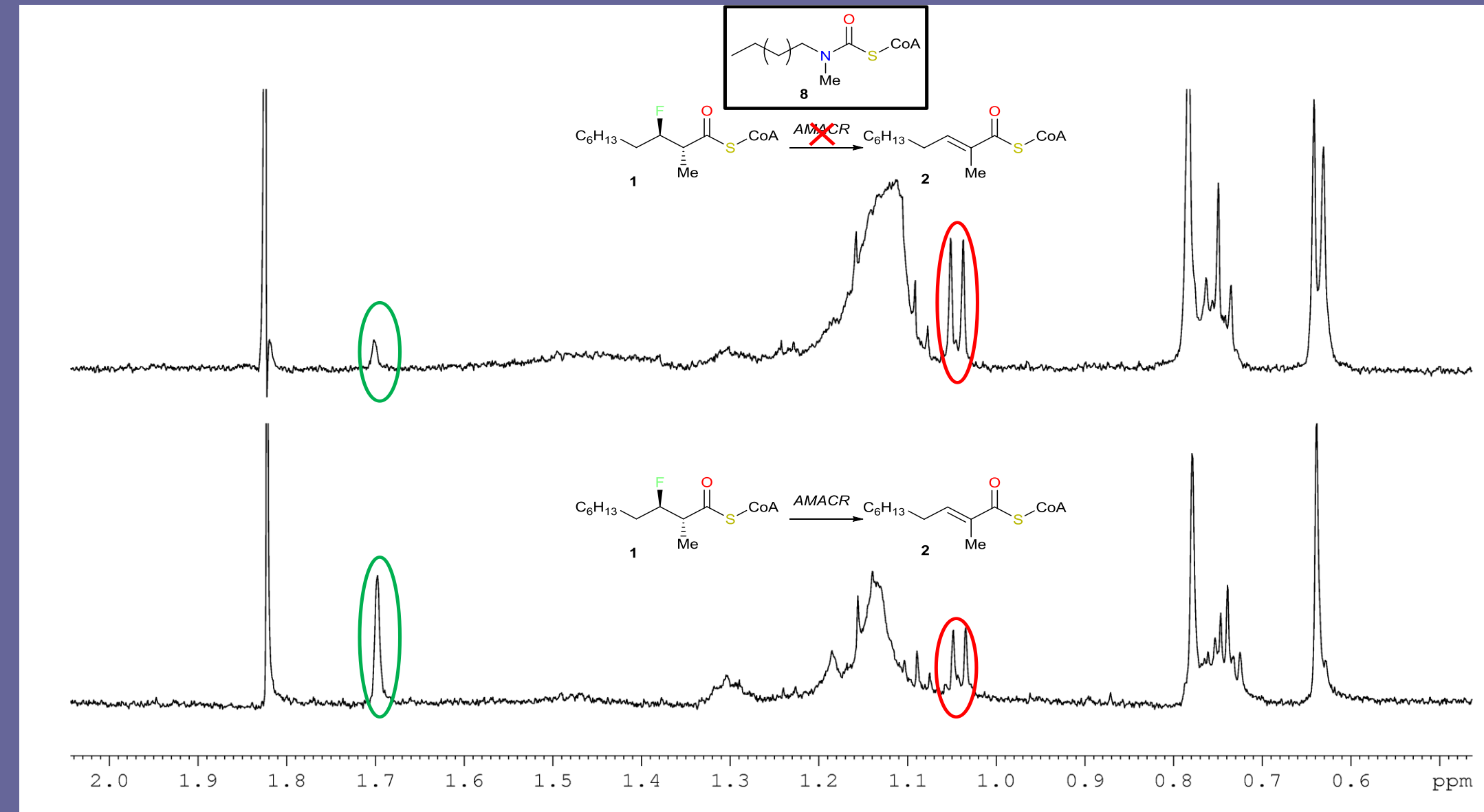
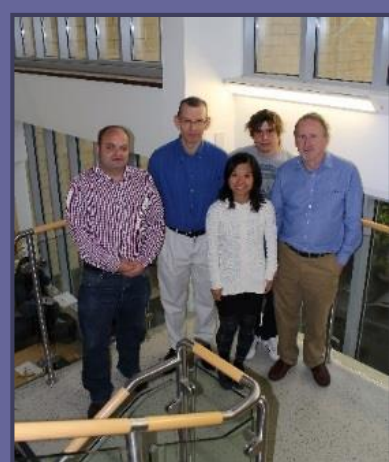


Figure 1: Reduction in conversion of **1** to **2** by the presence of **8**, as measuring the 2-Me group signal. A. No inhibitor; B. With 100 μ M **8**.

Synthesis of potential colorimetric substrates with aromatic side-chains was performed.³ The acyl-CoA substrate **12** was converted to the expected unsaturated product **13** by AMACR (Figure 2), but the resulting chromophore had a maximum wavelength of <340 nm. Conversion of the precursor acid to the acyl-CoA substrate was also difficult due to non-enzymatic elimination of the fluoride, and hence this approach was abandoned.

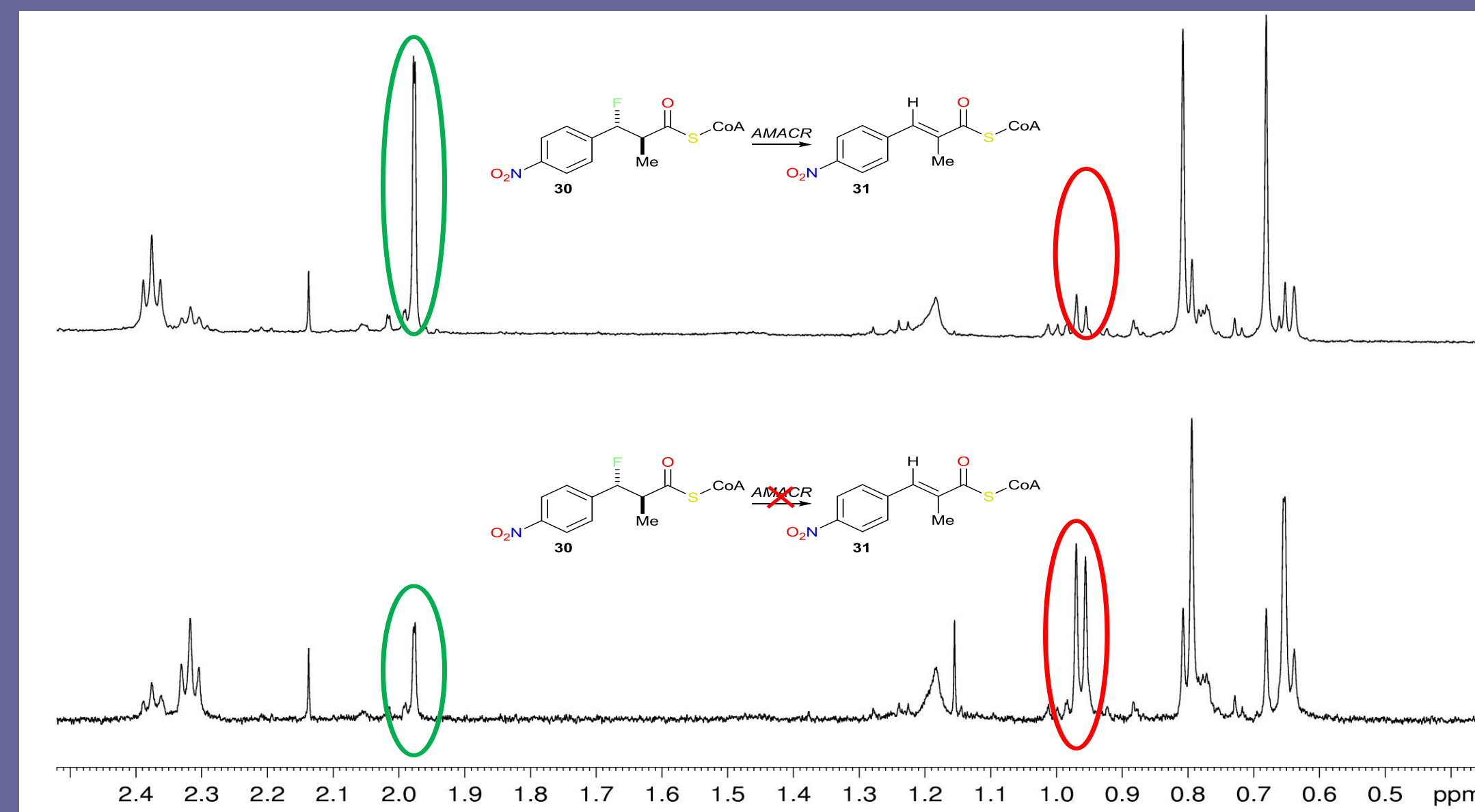


Figure 2: Conversion of aromatic substrate **12** to eliminated product **13**. A. Live enzyme; B. Negative control containing heat-inactivated enzyme.

Measurement of AMACR activity using fluoride sensors (Figure 3) was also investigated.³ Several sensors were chosen due to their apparent sensitivity and compatibility with aqueous environments. However, use of these sensors to measure AMACR activity proved to be technically difficult. Sensor **14**⁷ spontaneously decomposed in aqueous solution, generating a fluorescent signal in the absence of fluoride. Sensors **15**⁸ and **16**⁹ gave a better responses, but gave variable signal at the required fluoride concentrations in aqueous solution. Sensor **17**¹⁰ gave no fluorescent signal in the presence of fluoride, whilst sensor **18** could not be synthesised by literature methods.¹¹

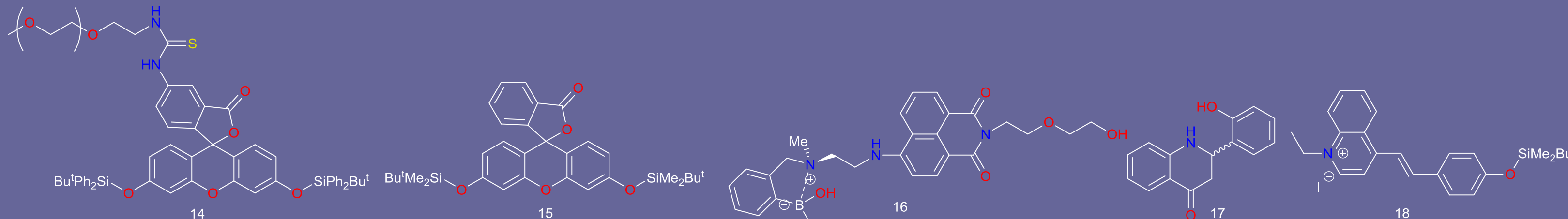


Figure 3: Fluoride sensors investigated to measure AMACR activity.

Conclusions

Conversion of 3-fluoro-2-methylacyl-CoA substrates by AMACR is reduced in the presence of inhibitors. However the stability of colorimetric substrates limits their use in AMACR activity assays. The investigated fluoride sensors lacked sufficient reproducibility and sensitivity for use in an assay.

Acknowledgements

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